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International application number: PCT/EP05/050465

International filing date: 03 February 2005 (03.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/541,368
Filing date: 04 February 2004 (04.02.2004)

Date of receipt at the International Bureau: 04 April 2005 (04.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

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**APPLICATION NUMBER:** ***60/541,368***
**FILING DATE:** ***February 04, 2004***

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Atty. Docket No.: P69474US0
**CUSTOMER NUMBER: 00136**



Mail Stop Provisional Patent Application
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Sir:

Transmitted herewith for filing is a PROVISIONAL APPLICATION of

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for **DIAGNOSTIC AND THERAPEUTIC USE OF KCNE4 PROTEIN FOR NEURODEGENERATIVE DISEASES.** The application comprises a 39 -page specification and 12 sheets of drawings.

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# DIAGNOSTIC AND THERAPEUTIC USE OF KCNE4 PROTEIN FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common neurodegenerative disease, accounting for about 70% of all dementia cases, and it is probably the most devastating age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles.

The amyloid-β protein evolves from the cleavage of the amyloid precursor protein (APP) by different kinds of proteases. The cleavage by the ß/γ-secretase leads to the formation of Aß peptides of different lengths, typically a short more soluble and slow aggregating peptide consisting of 40 amino acids and a longer 42 amino acid peptide, which rapidly aggregates outside the cells, forming the characteristic amyloid plaques (Selkoe, *Physiological Rev* 2001, 81: 741-66; Greenfield et al., *Frontiers Bioscience* 2000, 5: D72-83). Two types of plaques, diffuse plaques and neuritic plaques, can be detected in the brain of AD patients, the latter ones being the classical, most prevalent type. They are primarily found in the cerebral cortex and hippocampus. The neuritic plaques have a diameter of 50µm to 200µm and are composed of insoluble fibrillar amyloids, fragments of dead neurons, of microglia and astrocytes, and other components such as neurotransmitters, apolipoprotein E,

glycosaminoglycans, α1-antichymotrypsin and others. The generation of toxic Aß deposits in the brain starts very early in the course of AD, and it is discussed to be a key player for the subsequent destructive processes leading to AD pathology. The other pathological hallmarks of AD are neurofibrillary tangles (NFTs) and abnormal neurites, described as neuropil threads (Braak and Braak, *Acta Neuropathol* 1991, 82: 239-259). NFTs emerge inside neurons and consist of chemically altered tau, which forms paired helical filaments twisted around each other. Along the formation of NFTs, a loss of neurons can be observed. It is discussed that said neuron loss may be due to a damaged microtubule-associated transport system (Johnson and Jenkins, *J Alzheimers Dis* 1996, 1: 38-58; Johnson and Hartigan, *J Alzheimers Dis* 1999, 1: 329-351). The appearance of neurofibrillary tangles and their increasing number correlates well with the clinical severity of AD (Schmitt et al., *Neurology* 2000, 55: 370-376).

AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. The cognitive disturbances include among other things memory impairment, aphasia, agnosia and the loss of executive functioning. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-92).

The age of onset of AD may vary within a range of 50 years, with early-onset AD occurring in people younger than 65 years of age, and late-onset of AD occurring in those older than 65 years. About 10% of all AD cases suffer from early-onset AD, with only 1-2% being familial, inherited cases.

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon 4 allele of the three different existing alleles (epsilon 2, 3, and 4) of the apolipoprotein E gene (ApoE) (Strittmatter et al., *Proc Natl Acad Sci USA* 1993, 90: 1977-81; Roses, *Ann NY Acad Sci* 1998, 855: 738-43). The polymorphic plasmaprotein ApoE plays a role in the intercellular cholesterol and phospholipid transport by binding low-density lipoprotein receptors,

and it seems to play a role in neurite growth and regeneration. Efforts to detect further susceptibility genes and disease-linked polymorphisms, lead to the assumption that specific regions and genes on human chromosomes 10 and 12 may be associated with late-onset AD (Myers et al., *Science* 2000, 290: 2304-5; Bertram et al., *Science* 2000, 290: 2303; Scott et al., *Am J Hum Genet* 2000, 66: 922-32).
Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP) on chromosome 21, presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, agents, compositions, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

Voltage-gated potassium ion channels are trans-membrane proteins which consist of six transmembrane helices, and individual subunits homo- or hetero-tetramerize to form a functional ion channel. The main role of these so-called alpha-subunits is the regulation of the resting membrane of cells thereby regulating for instance neuronal excitability as well as cardiac action potential. The activity of such alpha-subunits may be regulated by interaction with intracellular soluble proteins or with transmembrane proteins consisting of a single transmembrane domain. Suc an interaction may lead to alterations of e.g. channel surface expression, gating kinetics or conduction properties.
The family of the potassium voltage-gated channel subfamily e (KCNE) genes is characterized by single-transmembrane proteins which interact mainly with voltage-gated potassium channels (Abbott and Goldstein, *Quart. Rev. Biophys.* 1998, 31: 357-359). The first member of this family was cloned in 1988 and interacts with an alpha-subunit which is important in shaping the cardiac action potential (Takumi et al., *Science* 1988, 242: 1042-1045). Subsequently, additional related peptides were cloned and characterized (Abbott et al., *Cell* 1999, 97: 175-187) among them being the potassium voltage-gated channel subfamily e member 4 (KCNE4). Th human

KCNE4 gene encodes 170 amino acids and is localized on chromosome 2q35-36 (Teng et al., *BBRC* 2003, 303: 808-813). The protein is highly homologous to KCNE4 from different species (e.g. 90% homology to mouse KCNE4). Northern blot analysis revealed strong expression of KCNE4 in heart, skeletal muscle and kidney. It is also expressed, albeit to a lesser extent, in placenta, lung and liver and to an even lesser extent in brain and blood cells (Teng et al., *BBRC* 2003, 303: 808-813). In addition, Grunnet et al. found expression of KCNE4 in embryonic tissue and in adult uterus (Grunnet et al., *J. Physiol.* 2002, 542.1: 119-130). Teng et al. and Grunnet et al. studied the biophysical properties of KCNE4. They found that this beta-subunit exerts its effects mainly on KCNQ1-potassium channels hereby inhibiting the ion-flux through the alpha-subunit. This inhibiting effect is not achieved by a redistribution of KCNQ1 from the plasma membrane to other cellular compartments. The activity of the highly homologous potassium channels KCNQ2-5 was not affected. KCNQ1 is expressed mainly in inner ear and in heart, and it has been described that mutations of KCNQ1 may lead to the so-called autosomal dominant Romano-Ward syndrome (which can lead to life threatening long QT-syndromes) and Jervell and Lange Nielsen syndrome which is a combination of congenital deafness and prolonged QT-intervals. However, the expression of KCNQ1 in brain awaits still verification whereas KCNE4-expression can readily be detected in brain. As the expression pattern of KCNE4 corresponds to the expression pattern of a different potassium ion channel, namely KCNJ2 or Kir2.1, Teng et al. speculate that KCNE4 might function as a modulator of the inwardly rectifying channel Kir2.1 (Teng et al., *BBRC* 2003, 303: 808-813). Abbott et al. were the first to clone the murine KCNE4 gene (Abbott et al., *Cell* 1999, 97: 175-187) and a subsequent patent application claims also the human counterpart for the diagnosis and treatment of cardiac arrhythmias (WO00/63434). The sequence of KCNE4 has also been dislosed in patent application WO99/55867.

The present invention discloses the detection and the differential regulation of KCNE4 gene expression in Alzheimer's disease brain samples versus age-matched control tissues of frontal cortex and of temporal cortex, respectively. To date, no experiments have been described that demonstrate a relationship between the dysregulation of KCNE4 gene expression and the pathology of neurodegenerative diseas s, in particular AD. Likewise, no mutations in the KCNE4 gene have been described to be associated with said diseases. Such a link, as disclosed in the

present invention, offers new ways, inter alia, for the diagnosis and treatment of said neurodegenerative diseas s, in particular AD.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as us d herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or oth rwise altered translation product. For the purpose of clarity, a derivative transcript, for instanc , refers to a transcript having

alterations in the nucleic acid sequence such as single or multiple nucleotide d letions, insertions, or exchanges. A d rivative translation product, for instance, may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition, or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. In the art, the terms "identity" and

"similarity" mean the degree of polypeptide or polynucleotide sequence relatedness which are determined by matching a query sequence and other sequences of preferably the same type (nucleic acid or protein sequence) with each other. Preferred computer program methods to calculate and determine "identity" and "similarity" include, but are not limited to GCG BLAST (Basic Local Alignment Search Tool) (Altschul et al., *J. Mol. Biol.* 1990, 215: 403-410; Altschul et al., *Nucleic Acids Res.* 1997, 25: 3389-3402; Devereux et al., *Nucleic Acids Res.* 1984, 12: 387), BLASTN 2.0 (Gish W., http://blast.wustl.edu, 1996-2002), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 1988, 85: 2444-2448), and GCG GelMerge which determines and aligns a pair of contigs with the longest overlap (Wilbur and Lipman, *SIAM J. Appl. Math.* 1984, 44: 557-567; Needleman and Wunsch, *J. Mol. Biol.* 1970, 48: 443-453). The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention, but retains its essential properties. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise a sequence that has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of KCNE4 protein, SEQ ID NO. 1. "Variants" include, for example, proteins with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention include variants, fragments and chemical derivatives of the protein comprising the amino acid sequences of KCNE4 protein, SEQ ID NO. 1. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. The term "isolated" as used herein is considered to refer to molecules or substances which have been changed and/or that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in natur . This notion further means that the sequenc s encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by

recombinant and/or synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those sequences are still present in said organisms, they are still considered to be isolated. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease.

The term "AD" shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see: Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer`s Disease and Related Disorders (Etiology, Pathogenesis and Therapeutics)*, Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer`s Disease*, Humana Press, Totowa, New Jersey, 2000; Mayeux and Christen, *Epidemiology of Alzheimer`s Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer`s Disease*, Springer Press, Berlin, Heidelberg, New York, 1998). The term "Braak stage" or "Braak staging" refers to the classification of brains according to the criteria proposed by Braak and Braak (Braak and Braak, *Acta Neuropathology* 1991, 82: 239-259). On the basis of the distribution of neurofibrillary tangles and neuropil threads, the neuropathologic progression of AD is divided into six stages (stage 0 to 6). The higher the Braak stage the more likely is the possibility to display the symptoms of AD. For a neuropathological assessment, i.e. an estimation of the probability that pathological changes of AD are the underlying cause of dementia, a recommendation is given by Braak H. (www. alzforum.org).

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for KCNE4 protein, and/or of (ii) a translation product of the gene coding for KCNE4 protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the gene coding for KCNE4 protein,

and/or of (ii) a translation product of the gene coding for KCNE4 protein, and/or of (iii) a fragment , or derivative, or variant of said transcription or translation product in a sample from said subject is determined. Said l vel and/or said activity is compared to a reference value representing a known disease or health status. Thereby, the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for KCNE4 protein, and/or of (ii) a translation product of the gene coding for KCNE4 protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said KCNE4 gene and protein, also referred to as minimum potassium ion channel-related peptide 3, MIRP3, or MINK-related peptide 3 protein, is represented by the gene coding for the proteins of Genbank accession numbers Q8WWG9 and/or Q96CC4 (protein IDs). The amino acid sequences of said proteins are deduced from the mRNA seqeunce corresponding to the cDNA sequence of Genbank accession number BC014429. In the instant invention KCNE4 also refers to the nucleic acid sequence of SEQ ID NO. 2, coding for the protein of SEQ ID NO. 1 (Genbank accession number Q8WWG9). In the instant invention said sequences are "isolated" as the term is employed herein. Further, in the instant invention, the gene coding for said KCNE4 protein is also generally referred to as the KCNE4 gene, or simply KCNE4, and the protein of KCNE4 is also generally referred to as the KCNE4 protein, or simply KCNE4.

In a further preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of th instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

The pres nt invention discloses the detection, differential expression and dysregulation of the gene coding for KCNE4 prot in in specific brain regions of AD patients in comparison to control persons. Further, the present invention discloses that the gene expression of KCNE4 is dysregulated in AD-affected brains, in that KCNE4 mRNA levels are elevated in the temporal cortex as compared to the frontal cortex of AD patients, whereas KCNE4 expression does not differ between the temporal and frontal cortex of healthy age-matched control subjects. Consequently, the KCNE4 gene and its corresponding transcription and translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, the gene coding for KCNE4 protein and its products may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is preferred that the sample to be analyzed and determined is selected from the group comprising brain tissue or other tissues, or body cells. The sample can also comprise cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or mucus. Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced *ex corpore*, and such methods preferably relate to samples, for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for KCNE4 protein, and/or of (ii) a translation product of the gene coding for KCNE4 protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an alteration in the level and/or activity of a transcription product of the gene coding for KCNE4 protein and/or of a translation product of the gene coding for KCNE4 prot in and/or of a fragment, or derivative, or variant ther of

in a sample cell, or tissue, or body fluid from said subject relative to a reference value repr senting a known health status indicat s a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

In preferred embodiments, measurement of the level of transcription products of the gene coding for KCNE4 protein is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based microarray technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

Furthermore, a level and/or an activity of a translation product of the gene coding for KCNE4 protein and/or of a fragment, or derivative, or variant of said translation product, and/or the level of activity of said translation product, and/or of a fragment, or derivative, or variant thereof, can be detected using an immunoassay, an activity assay, and/or a binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R, *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000).

In a preferred embodim nt, the level, or the activity, or both said level and said activity of (i) a transcription product of the gene coding for KCNE4 protein, and/or of (ii) a translation product of the gene coding KCNE4 protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the gene coding for KCNE4 protein (ii) reagents that selectively detect a translation product of the gene coding for KCNE4 protein; and

(b) instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the gene coding for KCNE4 protein, in a sample from said subject; and
- diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the pr sent invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerativ disease, in particular AD. Consequently, the kit, according to the present invention, may serve as a

means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflict d. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of the gene coding for KCNE4 protein, and/or (iii) a translation product of the gene coding for KCNE4 protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of the gene coding for KCNE4 protein, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the gene coding for KCNE4 protein, either in sense orientation or in antisense orientation.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents,

detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcription products of the gene coding for KCNE4 protein. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligo-deoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418: 244-251).

In a further preferred embodiment, a method to investigate the effects of compounds and/or agents on KCNE4 coexpressed with KCNQ1 or other potassium channels in appropriate cells, for example CHO cells or HEK293 cells, or other neuronal cell lines, is provided. Thereby, the electrophysiological effect of compounds and/or agents on the potassium current mediated by KCNE4 coexpressed with KCNQ1 or

with other potassium channels is examined. To conduct said examination the cDNA coding for human gene product KCNE4 is cloned into an appropriate xpression-vector. The cDNA coding for KCNQ1 (Genbank accession number U40990), or for other voltage-gated potassium channnels, is cloned into another appropriate expression-vector. Appropriate cell lines, as mentioned above, are transfected with said plasmids, preferably using a reagent like DMRIE-C (liposome formulation of the cationic lipid 1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide-chloesterol). Patch-clamp experiments can be performed in the voltage-clamp mode (Hamill et al., *Pflügers Arch.* 1981, 391: 85-100), and whole-cell currents will be recorded, and the obtained signals will be amplified, digitized, stored and analyzed using an appropriate software, for example Pulse/Pulsefit (HEKA, Lambrecht, Germany). If current "run-down" or "run-up" (Varnum *et al.*, *Pro. Natl. Acad. Sci. USA* 1993, 90: 11528-11532) remains to be too strong for compound and/or agent effect evaluation, investigations on the mediated currents of said voltage-gated potassium channels can be performed with the perforated patch-clamp method to prevent unspecific current amplitude changes (Dart et al., *J. Physiol.* 1995, 483: 29-39; Dinesh & Hablitz, *Brain Res.* 1990, 535: 318-322). An example of a stimulation protocol for the investigation of the effects and reversibility of test compounds on KCNE4 coexpressed with KCNQ1, or other potassium channels, is given below. Cells coexpressing KCNE4 with KCNQ1, or other potassium channels, will be clamped at a holding potential of e.g. -80 mV. The pulse cycling rate may be 10 s. For the compound and/or agent testing, stably transfected cells can be hyperpolarized from a holding potential of e.g. −80 mV for e.g. 100 ms to e.g. −90 mV, followed by, for instance, a 1s depolarization to +40 mV. The current amplitude at the end of the test pulse to +40 mV will be used for the analysis. The method is also suitable to identify and test compounds and/or agents which are capable for opening, closing, activating, inactivating, or modifying the biophysical properties of KCNE4 coexpressed with KCNQ1 or other potassium channels. Modulators of potassium channels, thus identified and tested, can potentially influence learning and memory functions and can be used for therapeutic approaches, for example for neurodegenerative diseases, in particular for Alzheimer's disease.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said

agent or gents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection or liposomal mediated transfection (see Mc Celland and Pardee, *Expression Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, MA, 1999).

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of th adult brain are fre of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of the gene coding for KCNE4 protein, and/or (iii) a translation product of the gene coding for KCNE4 protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of the gene coding KCNE4 protein, and/or (iii) a translation product of the gene coding for KCNE4 protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of the gene coding for KCNE4 protein, and/or (iii) a translation product of the gene coding for KCNE4 protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In on aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native KCNE4 gene sequence, or a fragment, or a derivative, or variant thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1994 and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of on or more substances selected from the group consisting of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of th gene coding for KCNE4 protein, and/or (iii) a translation product of th gene coding for KCNE4 protein,

and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the l vel, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the gene coding for KCNE4 protein, and/or (ii) a transcription product of the gene coding for KCNE4 protein, and/or (iii) a translation product of the gene coding for KCNE4 protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said symptoms of a neurodegenerative disease, and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses the gene coding for KCNE4 protein, or a fragment thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native KCNE4 protein gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the

modulator with a pharmaceutical carrier. However, said modulator may also b identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and KCNE4 protein, or a fragment, or derivative, or variant thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said KCNE4 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding a detectable, preferably a fluorescently labelled ligand to said containers, and (iv) incubating said KCNE4 protein, or said fragment, or derivative or variant thereof, and said compound or plurality of compounds, and said detectable, preferably fluorescently labelled ligand, and (v) measuring the amounts of preferably the fluorescence associated with said KCNE4 protein, or with said fragment, or derivative, or variant thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said KCNE4 protein, or said fragment, or derivative, or variant thereof. It might be preferred to reconstitute said KCNE4 translation product, or fragment, or derivative, or variant thereof into artificial liposomes to generate the corresponding proteoliposomes to determine the inhibition of binding between a ligand and said KCNE4 translation product. Methods of reconstitution of KCNE4 translation products from detergent into liposomes have been detailed (Schwarz et al., *Biochemistry* 1999, 38: 9456-9464; Krivosheev and Usanov, *Biochemistry-Moscow* 1997, 62: 1064-1073). Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of the gene coding for KCNE4 protein, or a fragment, or derivative, or variant thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for scr ening assays of the instant invention are described in the following patent applications: WO 96/13744, WO

98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of the gene coding for KCNE4 protein by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to KCNE4 protein, or to a fragment, or derivative, or variant thereof. Said screening assay comprises (i) adding a liquid suspension of said KCNE4 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a detectable, preferably a fluorescently labelled compound or a plurality of detectable, preferably fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said KCNE4 protein, or said fragment, or derivative, or variant thereof, and said detectable, preferably fluorescently labelled compound or detectable, preferably fluorescently labelled compounds, and (iv) measuring the amounts of preferably the fluorescence associated with said KCNE4 protein, or with said fragment, or derivative, or variant thereof, and (v) determining the degree of binding by one or more of said compounds to said KCNE4 protein, or said fragment, or derivative, or variant thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Also in this type of assay it might be preferred to reconstitute an KCNE4 translation product or a fragment, or derivative, or variant thereof into artificial liposomes as described in the present invention. Said assay methods may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to KCNE4 protein, or a fragment, or derivative, or variant thereof.

In one further embodiment, the pres nt invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of the gene coding for KCNE4 protein by the aforementioned binding assays

and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features a protein molecule shown in SEQ ID NO. 1, said protein molecule being a translation product of the gene coding for KCNE4, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, in particular Alzheimer's disease.

The present invention further features a protein molecule shown in SEQ ID NO. 1, said protein molecule being a translation product of the gene coding for KCNE4, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, in particular Alzheimer's disease.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the gene coding for KCNE4 protein, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, NY, 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-in-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, New York, 1999 and Edwards R., *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of the KCNE4 gene, or fragments, or derivatives, or variants thereof.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

FIGURES:

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of AD patients and

healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 discloses the initial identification of the differential expression of the gene coding for KCNE4 protein in a fluorescence differential display screen. The figure shows a clipping of a large preparative fluorescent differential display gel. PCR products from the frontal cortex (F) and the temporal cortex (T) of two healthy control subjects and six AD patients were loaded in duplicate onto a denaturing polyacrylamide gel (from left to right). PCR products were obtained by amplification of the individual cDNAs with the corresponding one-base-anchor oligonucleotide and the specific Cy3 labelled random primers. The arrow indicates the migration position where significant differences in intensity of the signals for a transcription product of the gene coding for KCNE4 protein derived from frontal cortex and from the temporal cortex of AD patients as compared to healthy controls exist. The differential expression reflects an up-regulation of KCNE4 gene transcription in the temporal and frontal cortices of AD patients compared to the cortices of control persons. Comparing the signals derived from temporal cortex and frontal cortex of healthy non-AD control subjects with each other, no difference in signal intensity, i.e. no altered expression level can be detected.

Figure 3 illustrates the verification of the differential expression of the human KCNE4 gene in AD brain tissues (P) versus healthy control brain tissue samples (C) by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex region of AD patients and of healthy, age-matched control persons ($P_{(F)}$ - $C_{(F)}$; Figure 3a) was performed by the LightCycler rapid thermal cycling technique. Likewise, samples from the temporal cortex region of AD patients and of control individuals ($P_{(T)}$ - $C_{(T)}$; Figure 3b) were compared. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for cyclophilin B, the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figures depict the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. The curves delineating the amplification kinetics of KCNE4 cDNAs are significantly separated during the exponential phase of the

amplification reaction, for both brain regions analyzed: (i) frontal cortex of a normal control individual in comparison to frontal cortex of an AD patient (Figure 3a), and (ii) temporal cortex of a normal control individual in comparison to temporal cortex of an AD patient (Figure 3b). This indicates a differential expression of the gene coding for KCNE4 in the analyzed brain regions of AD patients in comparison with healthy control persons.

Figure 4 illustrates the verification of the differential expression of the human KCNE4 gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and the temporal cortex (T) of AD patients (Figure 4a) and samples from the frontal cortex (F) and the temporal cortex of healthy, age-matched control individuals (Figure 4b) was performed by the LightCycler rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for cyclophilin B, the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figures depict the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of KCNE4 cDNA from the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction are juxtaposed (Figure 4b, arrowheads), whereas in Alzheimer's disease (Figure 4a, arrowheads) there is a significant separation of the corresponding curves, indicating a differential expression of the gene coding for KCNE4 in the respective analyzed brain regions, preferably a dysregulation of a transcription product of the human KCNE4 gene, or a fragment, or derivative, or variant thereof, in the temporal cortex relative to the frontal cortex.

Figure 5 discloses SEQ ID NO. 1, the amino acid sequence of the human KCNE4 protein. The full length human KCNE4 protein comprises 170 amino acids.

Figure 6 shows SEQ ID NO. 2, the nucleotide sequence of the human KCNE4 cDNA, comprising 1204 nucleotides.

Figure 7 depicts SEQ ID NO. 3, the nucleotide sequence of the 193 bp KCNE4 cDNA fragment, identified and obtained by differential display and subsequent cloning (sequence in 5' to 3' direction).

Figure 8 outlines the sequence alignment of SEQ ID NO. 3 to the nucleotide sequence of KCNE4 cDNA (SEQ ID NO. 2).

Figure 9 depicts the sequence alignment of the primers used for KCNE4 transcription level profiling by quantitative RT-PCR with the corresponding clippings of SEQ ID NO. 2.

Figure 10 shows a schematic alignment of SEQ ID NO. 3 with the KCNE4 cDNA (SEQ ID NO. 2). The open rectangle represents the open reading frame of the gene coding for KCNE4 protein, thin bars represent the 5' and 3' untranslated regions (UTRs).

The table in figure 11 lists KCNE4 gene expression levels in the frontal cortex of AD patients relative to the frontal cortex of healthy age-matched control persons in three AD patient/control person pairs, herein identified by internal reference numbers P011/C005, P016/C011, P019/C008 (ratio 3.80 to 11.39) and in the temporal cortex of AD patients relative to the temporal cortex of healthy age-matched control persons in three AD patient/control person pairs, herein identified by internal reference numbers P011/C005, P016/C011, P019/C008 (ratio 4.31 to 7.64). The values shown are calculated according to the formula described herein (see below). The scatter plot diagram visualizes individual values of the patient to control person regulation ratios, in frontal cortex samples (dots) and in temporal cortex samples (triangles), respectively.

The table in figure 12 lists KCNE4 gene expression levels in the temporal cortex relative to the frontal cortex in fifteen AD patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P017, P019, P031, P038, P040, P041, P042, P046, P047, P048 and twentyfour healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008, C012, C014, C025, C026, C027, C028, C029, C030, C031, C032, C033, C034, C035, C036, C037, C041, C042, DE02, DE03, DE05, DE07. For an upregulation in the temporal cortex, the values shown are calculated according to the formula described herein (see below) and in case of an upregulation in the frontal cortex the reciprocal values are calculated, respectively. The bar diagram visualizes individual natural

logarithmic values of the temporal to frontal cortex, ln(IT/IF), and of the frontal to temporal cortex regulation factors, ln(IF/IT), in differ nt Braak stages (0 to 6).

EXAMPLE I:

(i) Brain tissue dissection from patients with AD:

Brain tissues from AD patients and age-matched control subjects were collected, on average, within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Figure 1) and stored at –80 °C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were utilised to generate a melting curve with the LightCycler technology as described in the supplied protocol by the manufacturer (Roche).

(iii) cDNA synthesis and identification of differentially expressed genes by fluorescence differential display (FDD):

In order to identify changes in gene expression in different tissue, a modified and improved differential display (DD) screening method was employed. The original DD screening method is known to those skilled in the art (Liang and Pardee, *Science* 1995, 267:1186-7). This technique compares two populations of RNA and provides clones of genes that are expressed in one population but not in the other. Several samples can be analyzed simultaneously and both up- and down-regulated genes can be identified in the same experiment. By adjusting and refining several steps in the DD method as well as modifying technical parameters, e.g. increasing redundancy, evaluating optimized reagents and conditions for reverse transcription of total RNA, optimizing polymerase chain reactions (PCR) and separation of the

products th reof, a technique was developed which allows for highly reproducible and sensitive results. The applied and improved DD technique was described in detail by von der Kammer et al. (*Nucleic Acids Research* 1999, 27: 2211-2218). A set of 64 specifically designed random primers were developed (standard set) to achieve a statistically comprehensive analysis of all possible RNA species. Further, the method was modified to generate a preparative DD slab-gel technique, based on the use of fluorescently labelled primers. In the present invention, RNA populations from carefully selected post-mortem brain tissues (frontal and temporal cortex) of Alzheimer's disease patients and age-matched control subjects were compared.

As starting material for the DD analysis we used total RNA, extracted as described above (ii). Equal amounts of 0.05 µg RNA each were transcribed into cDNA in 20 µl reactions containing 0.5 mM each dNTP, 1 µl Sensiscript Reverse Transcriptase and 1x RT buffer (Qiagen), 10 U RNase inhibitor (Qiagen) and 1 µM of either one-base-anchor oligonucleotides $HT_{11}A$, $HT_{11}G$ or $HT_{11}C$ (Liang et al., *Nucleic Acids Research* 1994, 22: 5763-5764; Zhao et al., *Biotechniques* 1995, 18: 842-850). Reverse transcription was performed for 60 min at 37°C with a final denaturation step at 93°C for 5 min. 2 µl of the obtained cDNA each was subjected to a polymerase chain reaction (PCR) employing the corresponding one-base-anchor oligonucleotide (1 µM) along with either one of the Cy3 labelled random DD primers (1 µM), 1x GeneAmp PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$ (Applied Biosystems), 2 µM dNTP-Mix (dATP, dGTP, dCTP, dTTP Amersham Pharmacia Biotech), 5 % DMSO (Sigma), 1 U AmpliTaq DNA Polymerase (Applied Biosystems) in a 20 µl final volume. PCR conditions were set as follows: one round at 94°C for 30 sec for denaturing, cooling 1°C/sec down to 40°C, 40°C for 4 min for low-stringency annealing of primer, heating 1°C/sec up to 72°C, 72°C for 1 min for extension. This round was followed by 39 high-stringency cycles: 94°C for 30 sec, cooling 1°C/sec down to 60°C, 60°C for 2 min, heating 1°C/sec up to 72°C, 72°C for 1 min. One final step at 72°C for 5 min was added to the last cycle (PCR cycler: Multi Cycler PTC 200, MJ Research). 8 µl DNA loading buffer were added to the 20 µl PCR product preparation, denatured for 5 min and kept on ice until loading onto a gel. 3.5 µl each were separated on 0.4 mm thick, 6% polyacrylamide (Long Ranger)/ 7 M urea sequencing gels in a slab-gel system (Hitachi Genetic Systems) at 2000 V, 60W, 30 mA, for 1 h 40 min. Following completion of the electrophoresis, gels were scanned with a FMBIO II fluorescence-scanner (Hitachi Gen tic Systems), using the appropriate FMBIO II Analysis 8.0 software. A full-scale picture was printed,

differentially expressed bands marked, excised from the gel, transferred into 1.5 ml containers, overlayed with 200 µl sterile water and kept at –20°C until extraction.
Elution and reamplification of DD products: The differential bands w re extracted from the gel by boiling in 200 µl $H_2O$ for 10 min, cooling down on ice and precipitation from the supernatant fluids by using ethanol (Merck) and glycogen/sodium acetate (Merck) at -20°C over night, and subsequent centrifugation at 13.000 rpm for 25 min at 4°C. Pellets were washed twice in ice-cold ethanol (80%), resuspended in 10 mM Tris pH 8.3 (Merck) and dialysed against 10 % glycerol (Merck) for 1 h at room temperature on a 0.025 µm VSWP membrane (Millipore). The obtained preparations were used as templates for reamplification by 15 high-stringency cycles in 25-µl PCR mixtures containing the corresponding primer pairs as used for the DD PCR (see above) under identical conditions, with the exception of the initial round at 94°C for 5 min, followed by 15 cycles of: 94°C for 45 sec, 60°C for 45 sec, ramp 1°C/sec to 70°C for 45 sec, and one final step at 72°C for 5 min.
Cloning and sequencing of DD products: Re-amplified cDNAs were analyzed with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies) and ligated into the pCR-Blunt II-TOPO vector and transformed into *E.coli* Top10F' cells (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) according to the manufacturer's instructions. Cloned cDNA fragments were sequenced by commercially available sequencing facilities. The result of one such FDD experiment for the gene coding for KCNE4 protein is shown in Figure 2.

(iv) Confirmation of differential expression by quantitative RT-PCR:
Positive corroboration of differential KCNE4 gene expression was performed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratios of KCNE4 cDNAs from the temporal cortices of AD patients and of healthy age-matched control individuals, from the frontal cortices of AD patients and of healthy age-matched control individuals, and the ratios of KCNE4 cDNAs from the temporal cortex and frontal cortex of AD patients and of healthy age-matched control individuals, respectively, were determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the gene coding for KCNE4:
5'-TCATCCCGCCAAATTCTGA-3' and 5'-GGTTTGCACCCACCACTGA-3'.
PCR amplification (95°C and 1 sec, 56°C and 5 sec, and 72°C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 87.5°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 99 bp for the gene coding for KCNE4 protein was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'- TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with n visibl primer dimers. Agarose gel analysis of the PCR product showed one single band with the

expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number $C_t$ for the gene coding for KCNE4 protein and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortices of AD patients and of healthy control individuals, from temporal cortices of AD patients and of healthy control individuals, and cDNAs from the frontal cortex and the temporal cortex of AD patients and of control individuals, respectively, were analyzed in parallel and normalized to cyclophilin B. The $C_t$ values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \wedge ( (C_t \text{ value} - \text{intercept}) / \text{slope} ) \quad [\text{ng total brain cDNA}]$$

The values for frontal cortex KCNE4 cDNAs of AD patients (P) and control individuals (C), the values for temporal cortex KCNE4 cDNAs of AD patients (P) and of healthy control individuals (C), and the values for temporal and frontal cortex KCNE4 cDNAs, respectively, were normalized to cyclophilin B and the ratios were calculated according to formulas:

$$\text{Ratio} = \frac{\text{KCNE4 P temporal [ng] / cyclophilin B P temporal [ng]}}{\text{KCNE4 C temporal [ng] / cyclophilin B C temporal [ng]}}$$

$$\text{Ratio} = \frac{\text{KCNE4 P frontal [ng] / cyclophilin B P frontal [ng]}}{\text{KCNE4 C frontal [ng] / cyclophilin B C frontal [ng]}}$$

$$\text{Ratio} = \frac{\text{KCNE4 temporal [ng] / cyclophilin B temporal [ng]}}{\text{KCNE4 frontal [ng] / cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the AD patient to control person temporal cortex ratios, of the AD patient to control person frontal cortex ratios, and of the temporal to frontal ratios of AD patients and control persons, respectively, of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for the gene coding for KCNE4 protein to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the respective ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of such quantitative RT-PCR analysis for the gene coding for KCNE4 protein are shown in Figures 3 and 4.

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## CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising determining a level and/or an activity of

(i) a transcription product of the gene coding for KCNE4 protein, and/or

(ii) a translation product of the gene coding for KCNE4 protein, and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

3. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the gene coding for KCNE4 protein and (ii) reagents that selectively detect a translation product of the gene coding for KCNE4 protein, and

(b) an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the gene coding for KCNE4 protein in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation

product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of a neurodeg nerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

4. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i) a gene coding for KCNE4 protein, and/or

(ii) a transcription product of the gene coding for KCNE4 protein, and/or

(iii) a translation product of the gene coding for KCNE4 protein, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii).

5. A recombinant, non-human animal comprising a non-native gene sequence coding for KCNE4 or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

6. Use of the recombinant, non-human animal according to claim 5 for screening, testing, and validating compounds, agents, and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

7. An assay for scr ening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(I) a gene coding for KCNE4 protein, and/or

(ii) a transcription product of the gene coding for KCNE4 protein, and/or

(iii) a translation product of the gene coding for KCNE4 protein, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

8. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the gene coding for KCNE4 protein, and/or

(ii) a transcription product of the gene coding for KCNE4 protein, and/or

(iii) a translation product of the gene coding for KCNE4 protein, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative dis ase or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing th activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

9. The method according to claim 8 wherein said test animal and/or said control animal is a recombinant animal which expresses KCNE4, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native KCNE4 gene transcriptional control element.

10. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to KCNE4 protein, or to a fragment, or derivative, or variant thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said KCNE4 protein, or a fragment, or derivative, or variant thereof, to a plurality of containers;

(ii) adding a detectable, in particular a fluorescently labelled compound or a plurality of detectable, in particular fluorescently labelled compounds to be screened for said binding to said plurality of containers;

(iii) incubating said KCNE4 protein, or said fragment, or derivative, or variant thereof, and said detectable, in particular fluorescently labelled compound or detectable, in particular fluorescently labelled compounds;

(iv) measuring amounts of preferably the fluorescence associated with said KCNE4 protein, or with said fragment, or derivative, or variant thereof; and

(v) determining the degree of binding by one or more of said compounds to said KCNE4 protein, or said fragment, or derivative, or variant thereof.

11. A protein molecule, said protein molecule being a translation product of the gene coding for KCNE4, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

12. A protein molecule, said protein molecule being a translation product of the gene coding for KCNE4, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or

treating, or ameliorating a neurodegenerative disease, pr ferably Alzheimer's disease.

13. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for KCNE4, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell.

## ABSTRACT

The present invention discloses the differential expression of the gene coding for KCNE4 protein in specific brain regions of Alzheimer's disease patients. Based on this finding, the invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using the KCNE4 gene and its corresponding gene products. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

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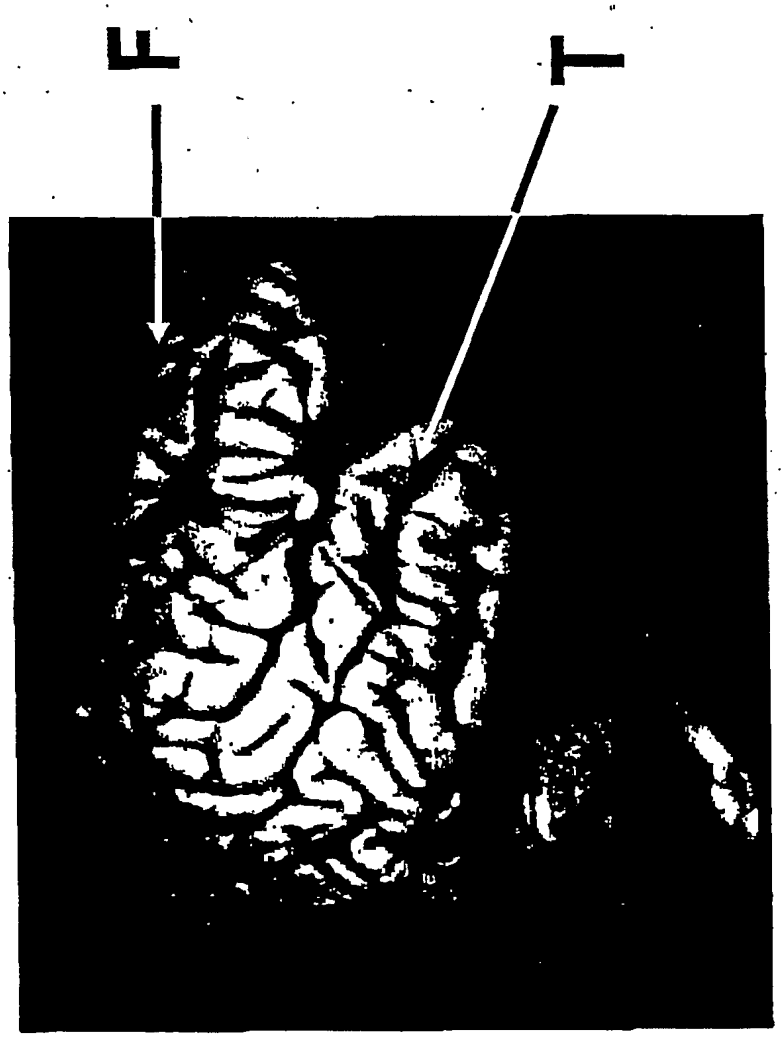


Fig. 1: Identification of Genes Involved in Alzheimer's Disease Pathology

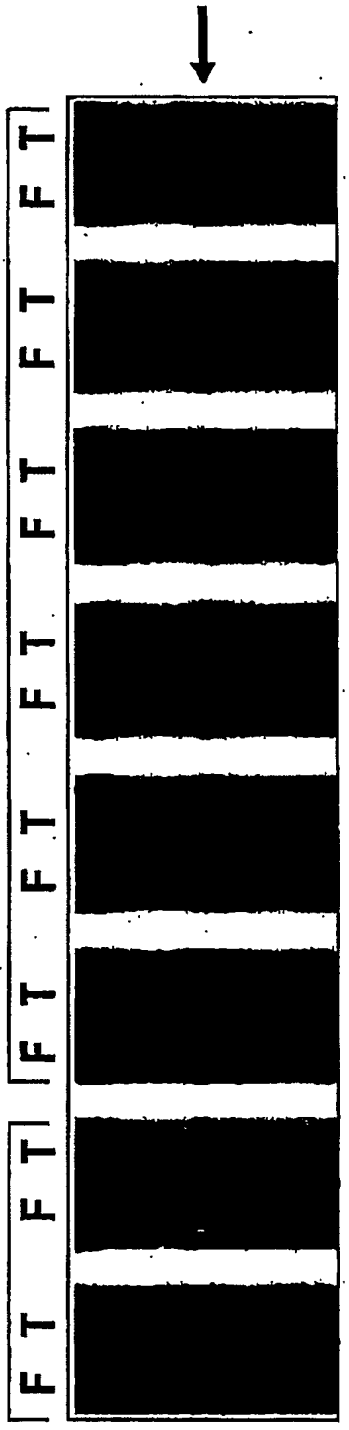


Fig. 2: Identification of differentially expressed genes in a fluorescence differential display screen

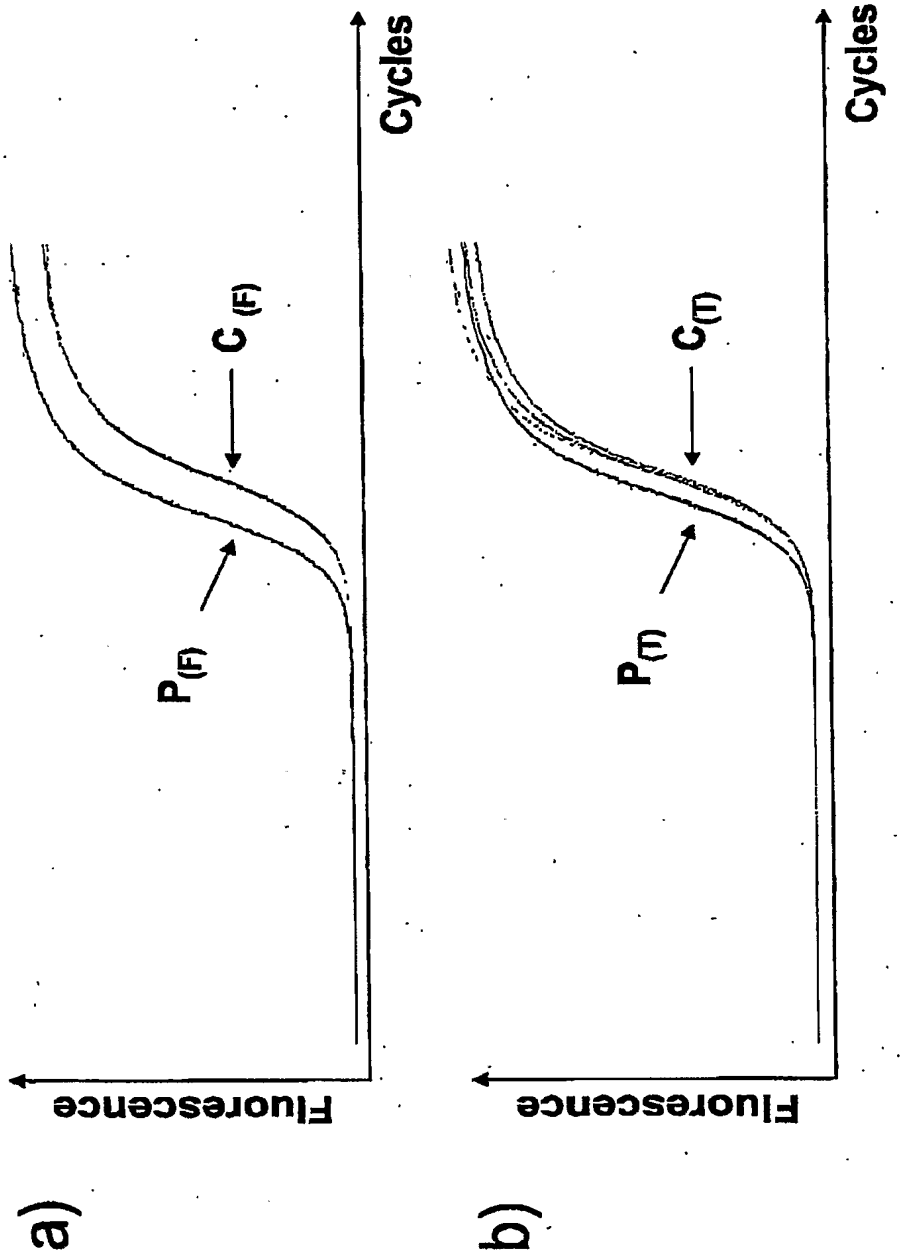


Fig. 3: Verification of differential expression of KCNE4 by quantitative PCR

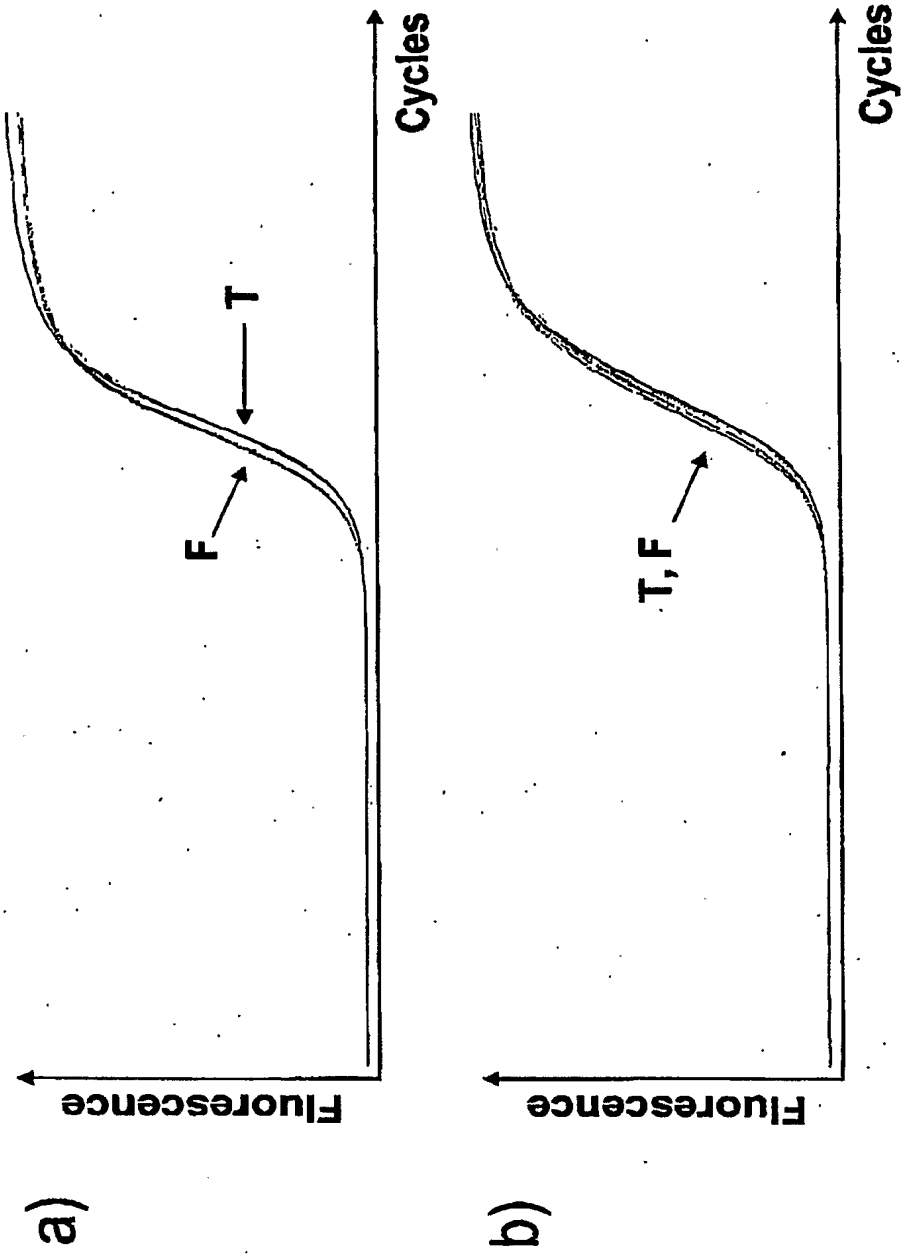


Fig. 4: Verification of differential expression of KCNE4 by quantitative PCR

**Fig. 5 : SEQ ID NO. 1:**
**amino acid sequence of**
**human KCNE4 protein**

**Length: 170 aa**

```
  1  MLKMEPLNST HPGTAASSSP LESRAAGGGS GNGNEYFYIL VVMSFYGIFL
 51  IGIMLGYMKS KRREKKSSLL LLYKDEERLW GEAMKPLPVV SGLRSVQVPL
101  MLNMLQESVA PALSCTLCSM EGDSVSSESS SPDVHLTIQE EGADEELEET
151  SETPLNESSE GSSENIHQNS
```

**Fig. 6: SEQ ID NO. 2:**
**nucle tide sequence f human KCNE4 cDNA**

**Length: 1204 bp**

```
   1  AGCAGAAGAA CCCTCTTGGA CTGGACGATT TGGGAATTCA AAACTTGGGA
  51  CAAACTGTCA GCCTTGCCCC TGCTGTGGAG GCAGCCTCAA TGCTGAAAAT
 101  GGAGCCTCTG AACAGCACGC ACCCCGGCAC CGCCGCCTCC AGCAGCCCCC
 151  TGGAGTCCCG TGCGGCCGGT GGCGGCAGCG GCAATGGCAA CGAGTACTTC
 201  TACATTCTGG TTGTCATGTC CTTCTACGGC ATTTTCTTGA TCGGAATCAT
 251  GCTGGGCTAC ATGAAATCCA AGAGGCGGGA GAAGAAGTCC AGCCTCCTGC
 301  TGCTGTACAA AGACGAGGAG CGGCTCTGGG GGGAGGCCAT GAAGCCGCTG
 351  CCCGTGGTGT CGGGCCTGAG GTCGGTGCAG GTGCCCCTGA TGCTGAACAT
 401  GCTGCAGGAG AGCGTGGCGC CCGCGCTGTC CTGCACCCTC TGTTCCATGG
 451  AAGGGGACAG CGTGAGCTCC GAGTCCTCCT CCCCGGACGT GCACCTCACC
 501  ATTCAGGAGG AGGGGGCAGA CGAGGAGCTG GAGGAGACCT CGGAGACGCC
 551  CCTCAACGAG AGCAGCGAAG GGTCCTCGGA GAACATCCAT CAGAATTCCT
 601  AGCACCCCCG GGACCCCTGC GGGTGGCTCC ATCAGCCAGC AACCTTAGAG
 651  AGAGGAAAGA CAGTTTTCAA GTGTCTGGTT TCACTTTCAC AGTGCGGCTG
 701  CCACTTTGAA GAGACCCTTG GTAAACCCCT GATTCGGGGT GGGGTGGGGG
 751  ACTAGGCTCA GCCGGAACCA GCACCTCCAA GGAGTCCGGG AGGTGCCTGT
 801  GGTTTGCACC CACCACTGAA AAAGCCGCGG AGATGCGCAG CGCGTACACT
 851  GACTTTGGGG CCTGGGTGTT GGGGTTCTGA TCAGAATTTG GCGGGATGAT
 901  ATGCTTGCCA TTTTCTCACT GGATGCCCTG GGTAGCTCCT GCAGGGTCTG
 951  CCTGTTCCCA GGGCTGCCGA ATGCTTAGGA CACGCTGAGA GACTAGTTGT
1001  GATTTGCTAT TTTGCCTAGA GCTTTGTCCT TCTAGATCTG ATTGGCTGTA
1051  AGTATCTCTA CTGTGTACCT GTGGCATTCC TTCACAGTGG GTTACAAGCT
1101  TCTTTTGGAT TAGAGGGGGA TTTTTGATGG GAGAAAGCTG GAGATCTGAA
1151  CCCAGCCCAT TTGCACACTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1201  AAAA
```

## Fig. 7: SEQ ID NO. 3

**Length: 193 bp**

```
  1  TAGTGTGCAA ATGGGCTGGG TTCAGATCTC CAGCTTTCTC CCATCAAAAA
 51  TCCCCCTCTA ATCCAAAAGA AGCTTGTAAC CCACTGTGAA GGAATGCCAC
101  AGGTACACAG TAGAGATACT TACAGCCAAT CAGATCTAGA AGGACAAAGC
151  TCTAGGCAAA ATAGCAAATC ACAACTAGTC TCTCAGCGTC ACC
```

## Fig. 8: Alignment of SEQ ID NO. 2 with SEQ ID NO. 3

**Length: 193 bp**

```
193 GGTGACGCTGAGAGACTAGTTGTGATTTGCTATTTTGCCTAGAGCTTTGT 144
    ||  ||||||||||||||||||||||||||||||||||||||||||||||
978 GGACACGCTGAGAGACTAGTTGTGATTTGCTATTTTGCCTAGAGCTTTGT 1027

143 CCTTCTAGATCTGATTGGCTGTAAGTATCTCTACTGTGTACCTGTGGCAT 94
    ||||||||||||||||||||||||||||||||||||||||||||||||||
1028 CCTTCTAGATCTGATTGGCTGTAAGTATCTCTACTGTGTACCTGTGGCAT 1077

93 TCCTTCACAGTGGGTTACAAGCTTCTTTTGGATTAGAGGGGGATTTTTGA 44
   |||||||||||||||||||||||||||||||||||||||||||||||||||
1078 TCCTTCACAGTGGGTTACAAGCTTCTTTTGGATTAGAGGGGGATTTTTGA 1127

43 TGGGAGAAAGCTGGAGATCTGAACCCAGCCCATTTGCACACTA 1
   |||||||||||||||||||||||||||||||||||||||||||
1128 TGGGAGAAAGCTGGAGATCTGAACCCAGCCCATTTGCACACTA 1170
```

**Fig. 9:** **Alignment of KCNE4 RT-PCR primers with human KCNE4 cDNA, SEQ ID NO. 2**

```
  1 GGTTTGCACCCACCACTGA 19
    ||||||||||||||||||
801 GGTTTGCACCCACCACTGA 819


 19 TCAGAATTTGGCGGGATGA 1
    ||||||||||||||||||
881 TCAGAATTTGGCGGGATGA 899
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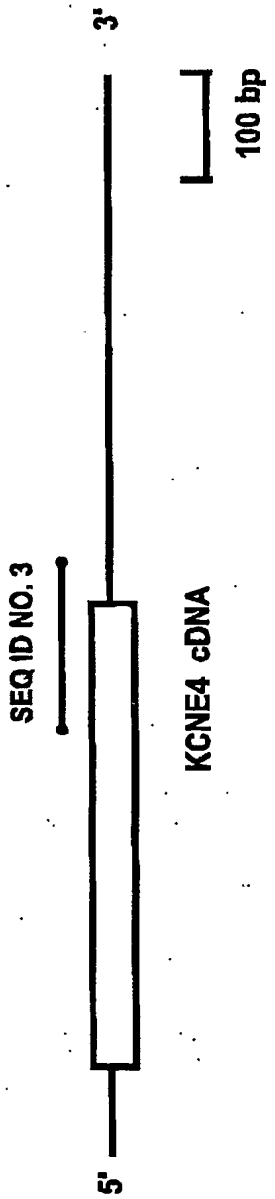


Fig. 10: Schematic alignment of SEQ ID NO. 3 with human KCNE4 cDNA, SEQ ID NO. 2

**Fig.11**

| sample | | ratio (patient / control person) |
|---|---|---|
| frontal cortex | P016/C011 | 7.65 |
| frontal cortex | P011/C005 | 11.39 |
| frontal cortex | P019/C008 | 3.80 |
| temporal cortex | P016/C011 | 7.64 |
| temporal cortex | P011/C005 | 5.23 |
| temporal cortex | P019/C008 | 4.31 |

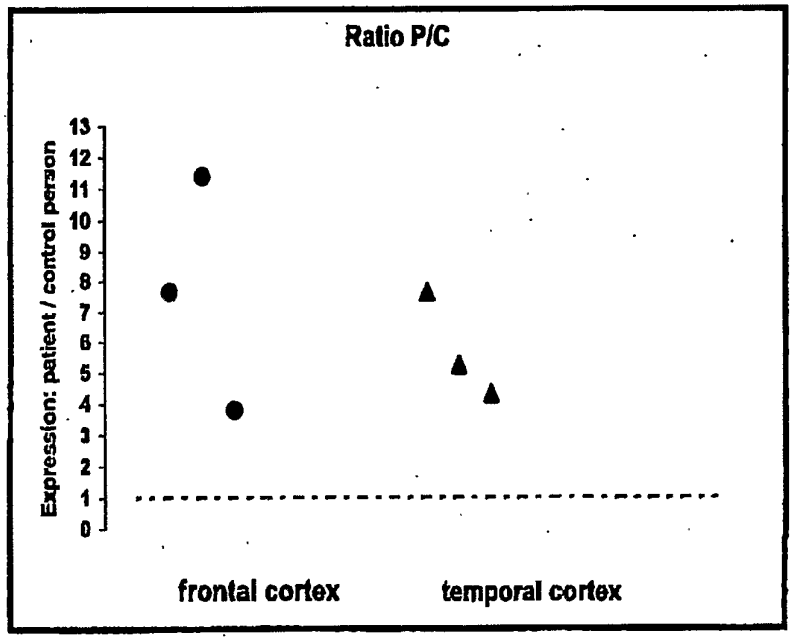

Fig.12